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Allozyme Variation of the Three Varieties of *Peucedanum japonicum* Thunb. in Japan

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Peucedanum japonicum comprises three allopatric varieties: var. *japonicum*, var. *latifolium*, and var. *australe*. Allozyme analysis was employed to estimate levels of genetic variation within and among populations of these varieties and to show whether the genetic differentiation is correlated with the three varieties. A UPGMA dendrogram defines three main clusters. These clusters are not concordant with the three varieties. Mean levels of expected heterozygosity showed that the Japanese mainland and the northern Ryukyus populations have low genetic variation and the central Ryukyus populations have high genetic variation. Genetic differentiation of *P. japonicum* may strongly reflect the geological history of the Ryukyu Islands.

Keyword: allozyme variation, *Peucedanum japonicum* var. *japonicum*, var. *latifolium*, var. *australe*, phytogeography, Ryukyu Islands

Peucedanum japonicum Thunb. is one of the common maritime plants in southern Japan and usually grows on rocks near the sea. Its geographic distribution ranges from; central Honshu and westward; Shikoku; south to Kyushu; the Ryukyu Islands; extending to the southern part of Korea; and into China; Taiwan; and the Batan Islands (Buwalda 1949, Hiroe & Constance 1958, She 1992).

Peucedanum japonicum has been considered a good species since Thunberg (1784) described it, due to the fact that *P. japonicum* is easily distinguished from other congeneric species by its entirely obovate leaflets. Recently Hotta & Shiuchi (1996) described the plants of the Tokara Islands as *P. japonicum* var. *latifolium* based on their higher plant height, wider leaflet and monocarpic life history. They also suggested that the *P. japonicum* distributed from Amami Island and southward would be of a different infraspecfic taxa. In our previous study (Seo & Hotta 2000), we analyzed geographical variation in four morphological characteristics (stem diameter, stem length, flowering shoot length and plant height) and two life history traits (flowering period and reproduction mode) of *P. japonicum* ranging from southern Kyushu to the Ryukyu Islands. This was in order to clarify the possible presence of distinctive features of infraspecies taxa within the complex. Based on the results, we divided *P. japonicum* into three varieties, var. *japonicum* (low plant height, flowering in autumn), var. *latifolium* (high plant height, flowering in summer) and var. *australe* (low plant height, flowering in summer). The difference between *P. japonicum* var. *japonicum* and var. *australe*, which have similar heights, is their leaflet shape (var. *japonicum* - not acute in apex, var. *australe* - slightly acute) as well as their flowering period. These differences may reflect a habitat difference as we shall discuss later.

According to Seo & Hotta (2000) and further field observation, geographical distribution of the three varieties is also differentiated; var. *japonicum* is distributed in western part of Kyushu, var. *latifolium* is in Honshu, Shikoku, Kyushu, Osumi Islands and Tokara Islands (excluding Takara Island), and var. *australe* is located from the southern Tokara Islands and further southward.

P. japonicum var. *latifolium* is characterized by its higher plant height. Many plants in the Tokara Islands were more than 100 cm tall, and the average plant height was the highest (about 200 cm) in Akuseki Island. The wide leaflet and monocarpic life history are also characteristics of var. *latifolium*.

Furthermore, the habitats of the three varieties are also differentiated. Var. *japonicum* grows on rocks near the sea. Var. *latifolium* grows along logging roads with *Miscanthus condensatus* and *Dicranopteris linearis* as well as on seashore. Var. *latifolium* often grows among tall plants while the other varieties grow in open habitats. This variety might have, through natural selection, adapted to grow more quickly from its seedling stage since only tall plants can get light in this habitat. Although var. *latifolium* requiers lower temperatures during winter for flower induction (Seo & Hotta 2000), temperature of Tokara Islands in winter is usually not low enough. This also may make the vegetative growth period of var. *latifolium* longer and the plants higher than the other two varieties. Habitat and temperature during winter would be main factor to influence plant size of var. *latifolium* on the Tokara Islands.

Many plants of var. *australe* often grow on Ryukyu limestone. Its habitat is very dry especially during the summer. It is possible that the narrower leaflet with a smaller leaf area may be an adaptation to the dry habitat.

Although ecological features and geographic distribution among the three varieties are well differentiated, the morphological characteristics mentioned above are continuous. Reproductive isolation may be incomplete, thus allowing for gene flow among the three varieties (Seo & Hotta 2000).

The objectives of the present study are 1) to investigate genetic differentiation among populations of *Peucedanum japonicum* complex in Japan; and 2) to show whether the genetic differentiation is correlated with the three varieties recognized by their morphological and ecological differentiation.

Materials and Methods

Plant material

A total of 718 separate plant samples were collected from 30 populations in Honshu, Shikoku, Kyushu and the Ryukyu Islands, covering the whole distribution of *Peucedanum japonicum* in Japan (Table 1, Fig. 1). Voucher specimens are deposited in the Herbarium of the Graduate School of Science, Kyoto University (KYO).

Allozyme analysis

About 100 mg of fresh leaf tissue was homogenized in a 1 ml extraction buffer (2 ml 2-mercaptoethanol, 20 mg polyvinyl-pyrrolidone, 0.1 mM Tris-HCl, 1 mM EDTA (4Na), 10 mM KCl, 10 mM $MgCl_2$, pH = 7.5). After centrifugation of homogenates at 10,000 rpm for 10 minutes at 10°C, 20 µl of supernatant per enzyme was used for electrophoresis for each enzyme. Vertical discontinuous polyacrylamide slab gel electrophoresis

TABLE 1. Locality infomation, sample size (N) used in the present study.
See Hotta & Shiuchi (1996), Seo & Hotta (2000) for morphlogical and ecological infomation.

Abbreviation		locality	N	leaf base	height	habitat	flower	reproduction mode
Peucedanum japonicum var. *japonicum*								
KYU-1	Kyushu	Kushikino	21	cuneate		no limestone	autumn	+
P japonicum var. *latifolium* M. Hotta & T. Shiuchi								
HON-1	Honshu	Tateyama city	30	cuneate	++	no limestone	summer	++
HON-2		Tateyama city	30	cuneate	++	no limestone	summer	++
HON-3		Miyake Island	27	cuneate	-	no limestone	-	-
HON-4		Irouzaki, Izu peninsula	15	cuneate	-	no limestone	-	-
HON-5		Takahama, Fukui	22	cuneate		no limestone	summer	+++
SHK	Shikoku	Ashizuri misaki	22	cuneate	-	no limestone	-	-
KYU-2	Kyushu	Uchinoura	25	cuneate	+	no limestone	summer	+++
KYU-3		Nagasakibana	32	cuneate, truncate	+	no limestone	summer	++
	Osumi Islands (the northern Ryukyus)							
OSU-1		Tane Island	27	truncate	-	no limestone	-	-
OSU-2		Tane Island	34	truncate	-	no limestone	-	-
OSU-3		Nagata, Yaku Island	25	truncate	++	no limestone	summer	++
OSU-4		Kurio, Yaku Island	25	truncate	++	no limestone	summer	++
	Tokara Islands (the northern Ryukyus)							
TOK-1		Suwanose Island	25	truncate	++	no limestone	autumn	+++
TOK-2		Akuseki Island	23	truncate	++	no limestone	summer	+++
P japonicum var. *australe* M. Hotta & A. Seo								
	Tokara Islands (the central Ryukyus)							
TOK-3		Oma, Takara Island	22	cuneate	+	limestone	summer	+++
TOK-4		Ogomori, Takara Island	25	cuneate	+	limestone	summer	+++
	Amami Islands (the central Ryukyus)							
AMA-1		Miyakozaki, Amami Island	18	cuneate	+	no limestone	summer	++
AMA-2		Toen, Amami Island	30	cuneate, truncate	++	no limestone	summer	+++
AMA-3		Waze, Amami Island	30	cuneate, truncate	+	no limestone	summer	++
AMA-4		Kinen, Toku Island	36	truncate	-	limestone	summer	-
AMA-5		Morogahama, Toku Island	23	truncate	-	no limestone	summer	-
AMA-6		Yoron Island	21	cuneate	+	limestone	summer	++
AMA-7		Yoron Island	13	cuneate	+	limestone	summer	++
	Okinawa Islands (the central Ryukyus)							
OKI-1		Imadomari, Okinawa Island	20	cuneate	+	limestone	summer	++
OKI-2		Zanpamisaki, Okinawa Island	23	cuneate	+	limestone	summer	++
	Sakishima Islands (the southern Ryukyus)							
SAK-1		Miyako Island	20	cuneate	-	limestone	-	-
SAK-2		Ishigaki Island	14	cuneate	-	limestone	-	-
SAK-3		Iriomote Island	32	cuneate	-	no limestone	-	++
SAK-4		Iriomote Island	29	cuneate	-	no limestone	-	++
			718					

-: no data
height: average value of population, + ≤ 100 cm, ++ > 100 cm
reproduction mode: +++, monocarpic only; ++, monocarpic dominant; +, polycarpic dominant

(Shiraishi 1988) was conducted for genotyping of all plant samples. The following 14 enzyme systems were examined: alcohol dehydrogenase (ADH; EC 1.1.1.1), diaphorase (DIA; EC 1.8.1.4), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), glutamate dehydrogenase (GDH; EC 1.4.1.2), glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), leuicine aminopeptidase (LAP; EC 3.4.11.1), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.40), 6-phosphogluconate dehydrogenase (6PG; EC 1.1.1.44), phosphoglucose mutase (PGM; EC 5.4.2.2), phosphoglucoisomerase (PGI; EC 5.3.1.9), shikimate dehydrogenase (SKD; EC 1.1.1.25), superoxide dismutase (SOD; EC 1.15.1.1). Staining procedures followed Shiraishi (1988) and Richardson *et al.* (1986) with minor modifications.

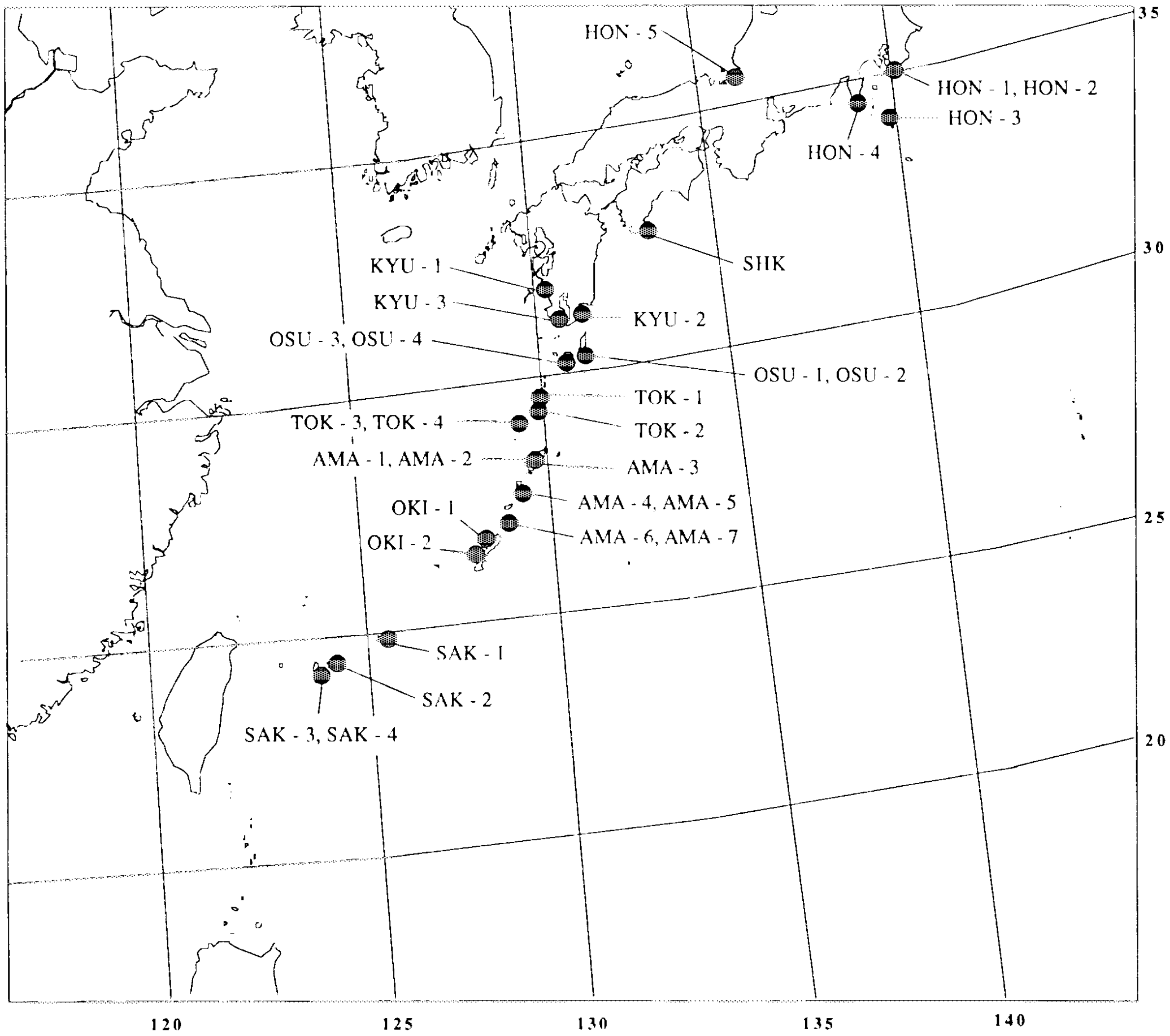

FIG. 1. Locality of *Peucedanum japonicum* populations. Abbreviations for populations are given in Table 1.

Loci were numbered in relation to their mobility; the longer distance zone was isozyme 1; the shorter distance zone was isozyme 2. Allele labels alphabetically denote relative mobility, the *a*-allele of each locus having the longest migrating band.

Data analysis

(1) Genetic variability within populations
Levels of allozyme diversity were estimated for all populations of *Peucedanum japonicum*. Alleles frequencies, the proportion of polymorphic loci (*P*), mean number of alleles per locus (*A*), and mean expected heterozygosities (*He*) were calculated using POPGENE program ver. 1.31 (Yeh *et al.* 1999).

(2) Genetic differentiation among populations
To estimate genetic differentiation among populations, *Fst* (Hartl & Clark 1989) and *Nm* estimated from *Fst* (Slatkin & Barton 1989) were calculated using the computer program POPGENE. Nei's (1972) genetic distance and genetic identity was calculated and a UPGMA dendrogram was constructed by using PHYLIP ver. 3.57 (Felsenstein 1995).

Results

Genetic variability within and among populations

Nine of 22 loci were polymorphic (*Adh-2*, *Dia-2*, *Got-1*, *Got-2*, *Lap*, *Pgm-1*, *Pgm-2*, *Pgm-3*, *Skd*). Five alleles were detected at *Lap*, *Got-2* and *Pgm-2*, four alleles at *Skd* and *Pgm-3*, three alleles at *Adh-2* and *Pgm-1*, and two alleles at *Dia-2*, *Got-1*. Allele frequencies at polymorphic loci for the 30 populations of *Peucedanum japonicum* are given in Table 2. Genetic distance and identity (Nei 1972) for pairwise varieties are presented in Table 3.

Levels of genetic variation within populations are presented in Table 4. The mean number of alleles per locus (*A*) ranged from 1.000 at HON-3, HON-5 and SAK-1 to 1.318 at AMA-2 and AMA-5. The *A* were lower at northern populations than in southern populations of *Peucedanum japonicum*. The same tendency was observed for the number of polymorphic loci per population. Mean levels of expected heterozygosity were significantly lower in var. *latifolium* than in var. *australe*. Expected heterozygosity was highest (0.079) in the Toen population of Amami Island (AMA-2). Expected heterozygosity in the populations of Takahama (HON-5) and Miyako Island (SAK-1) were 0.000, which had only monomorphic loci.

Geographic differentiation in allele frequencies were observed in several loci (Table 2). Both *Adh-2b* and *Dia-2a* were commonly observed in western Kyushu populations of *Peucedanum japonicum* var. *japonicum* as well as those of the Sakishima Islands, Okinawa Island, the Tokara Islands of vars. *latifolium* and *australe*. While *Adh-2c* and *Dia-2b* were commonly observed in Amami Islands populations (AMA-1, AMA-2, AMA-3, AMA-4, AMA-5, AMA-6, AMA-7) of var. *australe*. *Got-2b* was found commonly in the Amami Islands populations and rarely in Kyushu and the Osumi Islands populations. In var. *australe*, *Got-2e* was restricted to three Sakishima Islands populations (SAK-2, SAK-3, SAK-4). *Skd-a* was also observed commonly in Sakishima Islands populations (SAK-2, SAK-3, SAK-4) and rarely found on Yoron Island (AMA-6, AMA-7).

Genetic divergence

A dendrogram based on Nei's genetic distance is shown in Fig. 2. The dendrogram defines three main clusters: cluster I is composed of 19 populations of vars. *japonicum*, *latifolium* and *australe* from Honshu, Shikoku, Kyushu, the Osumi Islands, the Tokara Islands and Okinawa Island; cluster II is composed of 8 populations of var. *australe* from the Amami Islands and Miyako Island; cluster III is composed of 3 populations var. *australe* from the Sakishima Islands excluded Miyako Island. In Cluster I, 2 subclusters were defined; Subcluster I-1 is composed of 1 population of var. *japonicum* from western Kyushu, whereas subcluster I-2 is composed of 18 populations of vars *latifolium* and *australe*.

Fst were calculated for the population groups defined by Nei's genetic distances (Table 5). *Fst* values are highest in cluster II, and are lowest in cluster III. Within each cluster, estimated gene flow (*Nm*) from *Fst* values (Slatkin & Barton 1989) varied from 0.138 for cluster II to 0.319 for cluster III.

Discussion

Mean genetic identities (I = 0.892) of *Peucedanum japonicum* in Japan exhibit slightly lower value than those reported for plant conspecific populations (I = 0.95; Gottlieb 1981). Genetic identity values have previously been reported among intraspecific taxa of *Cheno-*

TABLE 2. Allele frequencies for 9 polymorphic allozyme loci. Population abbreviation correspond to Table 1.

		var. *japonicum*	var. *latifolium*														var. *australe*														
Locus	Allele	KYU-1	HON-1	HON-2	HON-3	HON-4	HON-5	SHK	KYU-2	KYU-3	OUS-1	OSU-2	OSU-3	OSU-4	TOK-1	TOK-2	TOK-3	TOK-4	AMA-1	AMA-2	AMA-3	AMA-4	AMA-5	AMA-6	AMA-7	OKI-1	OKI-2	SAK-1	SAK-2	SAK-3	SAK-4
Adh-2	a																													0.02	
	b	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.14	0.02				0.12	0.12	1.00	0.39		1.00	0.98	1.00
	c							0.07											0.86	0.98	1.00	1.00	1.00	0.88	0.88		0.61	1.00			
Dia-2	a	0.79	0.77	0.75	1.00	0.50	1.00	1.00	1.00	1.00	0.98	1.00	0.70	0.80	0.96	0.70	1.00	1.00	0.03	0.67		0.79	0.72		0.08	1.00	0.24		1.00	1.00	0.76
	b	0.21	0.23	0.25		0.50					0.02		0.30	0.20	0.04	0.30			0.97	0.33	1.00	0.21	0.28	1.00	0.92		0.76	1.00			0.24
Got-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b									0.08			0.10																		
Got-2	a																				0.58		0.07								
	b	0.14											0.06						1.00	1.00	0.42	1.00	0.93								
	c	0.86	1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00	0.96	0.93	0.94	1.00	0.98	1.00	1.00	1.00						1.00	1.00	1.00	1.00				
	d								0.10		0.04	0.07			0.02													1.00			
	e																												1.00	1.00	1.00
Lap	a																			0.20			0.02							0.02	0.07
	b							0.30		0.03												1.00	0.72			0.03				0.67	0.81
	c		1.00	1.00	1.00	1.00	1.00	0.61	1.00	0.88	1.00	1.00	0.76	1.00	0.60	1.00	0.30	0.20	0.78	0.55	1.00		0.24	1.00	1.00	0.68	1.00	1.00	0.64	0.31	0.12
	d	0.74						0.09		0.09			0.24		0.40		0.70	0.80	0.22	0.25			0.02			0.30			0.36		
	e	0.26																													
Pgm-1	a																	0.22											1.00	1.00	1.00
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.78	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
	c																														
Pgm-2	a																						0.26								
	b																		0.03												
	c																		0.72	0.58						0.20					
	d							1.00			1.00	1.00			1.00	1.00	0.45	0.54	0.25	0.13	1.00	0.01	0.59	1.00	1.00			1.00	1.00	1.00	
	e	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00			1.00	1.00			0.55	0.46		0.28		0.99	0.15			0.80	1.00				1.00
Pgm-3	a									0.05																					
	b																			0.03											
	c		1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	0.89	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	d	1.00																				0.11									
Skd	a																							0.02	0.04				1.00	0.80	0.97
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	0.91	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	0.98	0.88	0.03	0.39	1.00		0.20	0.03
	c										0.07	0.09		0.04							0.08										
	d																								0.08	0.98	0.61				

Locus labeles denote relative mobility: 1, faster zone; 2, midium zone; 3, slower zone
Allele labels were designated alphabetically from faster mobility to slower.

TABLE 3. Mean Nei's genetic identity between varieties by Seo & Hotta (2000) and among total populations

	japonicum	*latifolium*	*australe*
japonicum	-	0.901	0.825
latifolium		0.972	0.88
australe			0.855
mean	0.892		

podium incanum (*I* = 0.95; Crawford 1979), *Coreopsis cyclocarpa* (*I* = 0.75; Crawford & Bayer 1981), *Plantago major* (*I* = 0.99; Dijk & Delden 1981), *Solanum heterodoxum* (*I* = 0.83; Whalen 1979), *S. citrallifolium* (*I* = 0.71; Whalen 1979), and others (Crawford 1983). Mean genetic identity values among intraspecific taxa of *P. japonicum* were almost the same or slightly lower (var. *japonicum* versus var. *latifolium*, *I* = 0.901; var. *japonicum* versus var. *australe*, *I* = 0.825; var. *latifolium* versus var. *australe*, *I* = 0.880). Genetic identity values of intraspecific taxa of *P. japonicum* were similar to those values for conspecific species, *I* = 0.69 - 1.00 (*I* >0.90) and for subspecies and variety, *I* = 0.71-0.99 (reviewed in Crawford 1983).

A dendrogram generated from UPGMA clustering of genetic distance values using populations of *Peucedanum japonicum* as OTUs revealed three distinct groups of populations, clusters I - III (Fig. 2). These groups are not concordant with infraspecific taxa based on morphological and ecological data (Hotta & Shiuchi 1996; Seo & Hotta 2000). Geographically neighboring populations formed each cluster.

Var. *japonicum* and var. *latifolium* are included only in cluster I. Var. *australe* was scattered among the three clusters. In particular, var. *australe* from the southern Tokara Islands (TOK-3, TOK-4) and Okinawa Island (OKI-1, OKI-2) was genetically more similar to var. *japonicum* and var. *latifolium* than to the other populations of var. *australe*. Var. *australe* is defined by a narrower shaped leaflet and their habitat (growing on Ryukyu limestone) (Seo & Hotta 2000). The plants on the southern Tokara Islands and Okinawa Island have been identified as var. *australe* according to these traits. However it was shown that var. *australe* is genetically heterogenous and the morphological and ecological characteristics do not reflect the biological entity of var. *australe*. This suggests that morphological and ecological traits of *P. japonicum* of the southern Tokara Islands and Okinawa Island might have converged.

Okinawan populations of var. *australe* (OKI-1, OKI-2) were included in cluster I and separated from the Amami populations which formed cluster II (Fig. 2). The Okinawan populations have both alleles which dominate in the northern populations such as *Adh-2b* and those which dominate in the southern populations such as *Adh-2c* (Fig. 3). It can be concluded that the Okinawan populations were included in cluster I because frequency of the northern allele was higher in these populations.

The Takara Island populations (TOK-3, TOK-4) were classified as var. *australe* based on their morphology (Seo & Hotta 2000). However, allozyme analysis showed that these populations are genetically more similar to var. *latifolium*. The plants of Takara Island were reported to be mostly monocarpic (Seo & Hotta 2000). Monocarpic plants are dominant or often 100 % of the population of var. *latifolium*,

TABLE 4. Comparison of genetic variabilities in teams of the three measures in populations; *A*, mean number of alleles per locus; *P*, the proportion of polymorphic loci; *He*, mean expected heterozygosities

	A (s. d.)	*P*	*He* (s. d.)
P. japonicum var. *japonicum*			
KYU-1	1.136 (0.351)	0.136	0.045 (0.118)
P. japonicum var. *latifolium*			
HON-1	1.046 (0.213)	0.045	0.017 (0.081)
HON-2	1.046 (0.214)	0.045	0.017 (0.078)
HON-3	1.000	0.000	0.000
HON-4	1.046 (0.213)	0.045	0.024 (0.110)
HON-5	1.000	0.000	0.000
SHK	1.136 (0.468)	0.091	0.031 (0.117)
KYU-2	1.046 (0.213)	0.045	0.008 (0.039)
KYU-3	1.182 (0.501)	0.136	0.021 (0.059)
OSU-1	1.136 (0.351)	0.136	0.011 (0.033)
OSU-2	1.091 (0.294)	0.091	0.014 (0.045)
OSU-3	1.182 (0.395)	0.182	0.050 (0.122)
OSU-4	1.091 (0.294)	0.091	0.018 (0.071)
TOK-1	1.136 (0.351)	0.136	0.028 (0.104)
TOK-2	1.046 (0.213)	0.045	0.020 (0.092)
mean	1.085 (0.300)	0.078	0.019 (0.077)
P. japonicum var. *australe*			
TOK-3	1.091 (0.294)	0.091	0.042 (0.138)
TOK-4	1.134 (0.351)	0.136	0.054 (0.142)
AMA-1	1.227 (0.528)	0.182	0.049 (0.123)
AMA-2	1.318 (0.646)	0.227	0.079 (0.191)
AMA-3	1.091 (0.294)	0.091	0.030 (0.109)
AMA-4	1.136 (0.351)	0.136	0.026 (0.081)
AMA-5	1.318 (0.780)	0.182	0.071 (0.169)
AMA-6	1.091 (0.294)	0.091	0.012 (0.046)
AMA-7	1.182 (0.501)	0.136	0.026 (0.069)
OKI-1	1.182 (0.501)	0.136	0.038 (0.118)
OKI-2	1.136 (0.351)	0.136	0.061 (0.159)
SAK-1	1.000	0.000	0.000
SAK-2	1.046 (0.213)	0.045	0.022 (0.102)
SAK-3	1.182 (0.501)	0.136	0.037 (0.117)
SAK-4	1.182 (0.501)	0.136	0.035 (0.104)
mean	1.154 (0.444)	0.124	0.039 (0.118)
total mean	1.121 (0.146)	0.103	0.03 (0.101)

whereas at least 15-20 % of the plants of var. *australe* populations are polycarpic (Seo & Hotta 2000). This showed that ecological traits of plants on Takara Island are more similar to that of var. *latifolium* than var. *australe*. Furthermore, *Adh-2c* allele was observed in most populations of var. *australe* but only *Adh-2b* was observed in the two populations of Takara Island included in this study. This might show that plants with *Adh-2b* allele migrated from Amami Island to Takara Island and recolonized. However, *Got-2b* allele which was a unique allele in the Amami Islands populations was not found in Takara populations. This refutes the hypothesis of a migation from Amami Island. The southern Tokara Islands are the northernmost limit of the distribution of Ryukyu limestone (Kimura 2000). The plants of Toku, Yoron and Miyako Islands grow on Ryukyu limestone. It may be surmised that var. *latifolium* plants on Takara Island became smaller in size and narrower in leaflet shape through adaptation to an open limestone habitat.

The Sakishima Islands populations (SAK-2, SAK-3, SAK-4) had several unique alleles and diverged from other populations (Table 2, Fig. 2). Geographic patterns of amphibians and reptiles of the Ryukyu Islands showed a large faunal gap between Sakishima Islands (the southern Ryukyus) and Amami and Okinawa Islands (the central Ryukyus) (Ota 1998, 2000). Genetic differentiation in a frog species, *Rana limnocharis*, showed similar vicariance between the southern Ryukyus and the surrounding area (Toda *et al.* 1997). Toda *et al.* (1997) suggested that this differentiation occured due to the fact that the southern Ryukyus may have been isolated at least twice, during the Pliocene and again during the Pleistocene Ages (Kizaki & Oshiro 1980). Genetic differentiation of *P. japonicum* of the southern Ryukyus might also result from the same geographical isolation.

Miyako Island is geographically classifed as part of the Sakishima Islands but its population of *Peucedanum japonicum* (SAK-1) was more genetically similar to the Amami Island populations than the other Sakishima Island populations (Fig. 2). The *Trimeresurus* species (Habu snake) of the Ryukyu Islands is distributed from the southern Tokara Islands to Ishigaki and Iriomote Islands, excepting Okinoerabu, Yoron and Miyako Islands (Koba

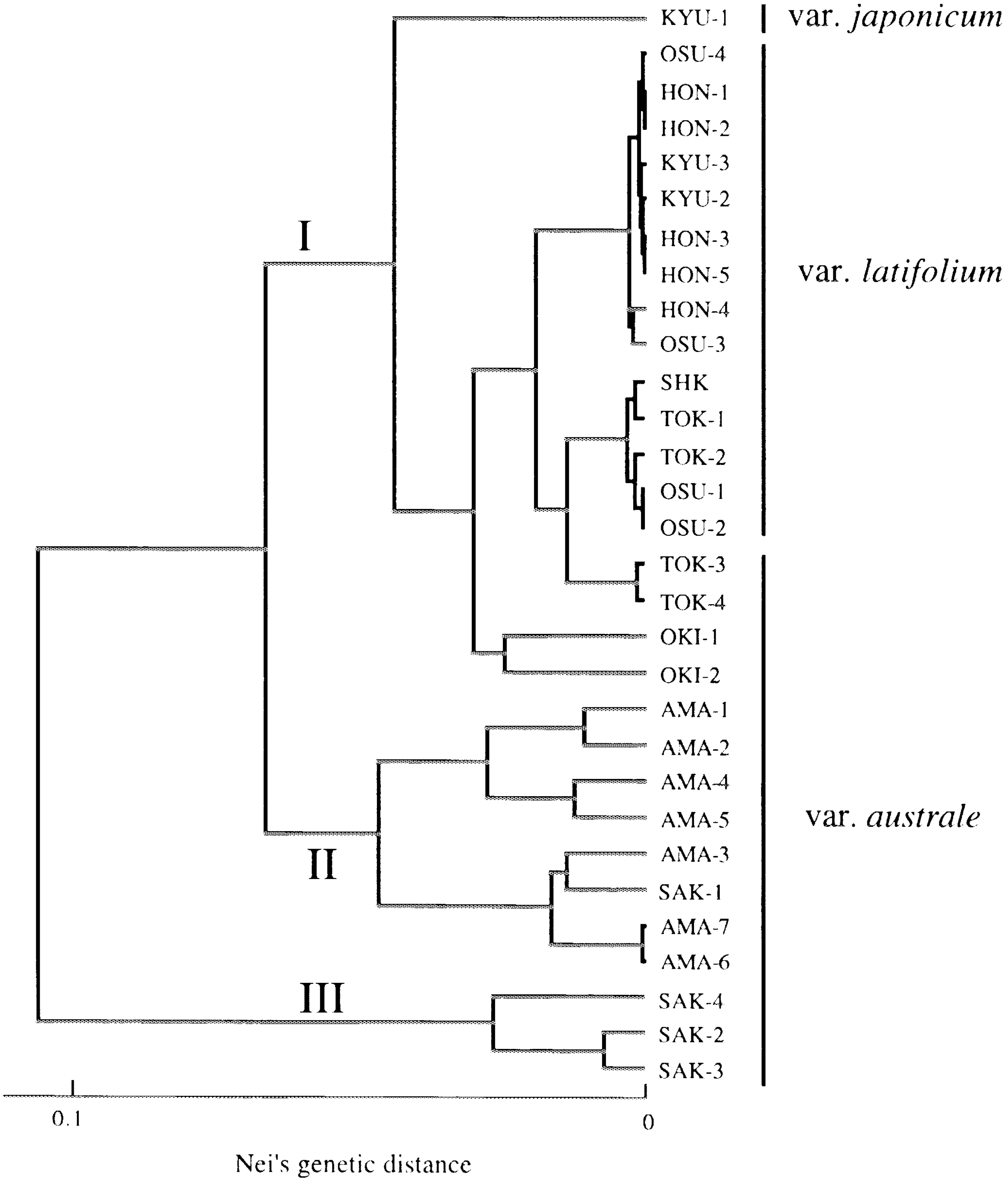

FIG. 2. UPGMA dendrogram of *Peucedanum japonicum* based on Nei's genetid distance. Abbreviations for populations are given in Table 1.

1971). Hanzawa (1935) dicussed that *Trimeresurus* had once been distributed on the latter three islands, but these venomous snakes became extinct when these islands were submerged. He considered that all land biota of Miyako Island became extinct at one time. Thus *P. japonicum* growing on Miyako Island would also have become extinct at that time and then immigrated from the Amami Islands. The lack of genetic diversity of the Miyako Island

TABLE 5. F statistics and estimated number of migration per generation (Nm) calculated within and among the three clusters.

	Fst	Nm
Cluster I	0.617	0.156
Cluster II	0.644	0.138
Cluster III	0.439	0.319
all populations	0.778	0.071

population might also support this hypothesis.

The mean values of A, P, He of *Peucedanum japonicum* were lower than those reported for gymnosperms ($A = 1.93$, $P = 0.577$, $He = 0.16$) and dicots ($A = 1.44$, $P = 0.29$, $He = 0.096$) (Hamrick & Godt 1990). Furthermore, the values of *P. japonicum* were lower than those reported for plants distributed in temperate-tropical regions ($A = 1.52$, $P = 0.359$, $He = 0.123$) and for selfing and outcrossing plants ($A = 1.31$ and 1.64, $P = 0.20$ and 0.412, $He = 0.074$ and 0.133, respectively) (Hamrick & Godt 1990). This shows that the genetic diversity of *P. japonicum* is slightly lower than other plant species.

The mean values of A, P of var. *australe* were the highest among three varieties. The value of He of var. *japonicum* was the highest among three varieties. However, He of 5 of 15 populations of var. *australe* were higher than the value of var. *japonicum*. Populations having higher genetic diversity are distributed in the southern part of the Tokara Islands, the Amami and Okinawan Islands.

The mean value of He of var. *latifolium* was the lowest among three varieties. This shows that genetic diversity of var. *latifolium* is the lowest among the three varieties. Var. *latifolium* have almost the same alleles at many loci and this variety is more genetically homogenous than vars. *japonicum* and *australe*. Plant height of var. *latifolium* was reported to be more variable than the other two varieties (Seo & Hotta 2000). High genetic identity and low genetic diversity among populations of var. *latifolium* may show rapid morphological differentiation.

The mean value of A, P and He of var. *australe* was higher than that of var. *latifolium*. 8 of 33 alleles at 9 polymorphic loci were specific for var. *australe*. This reflects the high genetic diversity of var. *australe*. Var. *australe* on the Amami and Toku Island populations (AMA-2 and AMA-5) had the highest genetic diversities (A, P, He) among whole populations. Amami and Toku Islands are relatively larger islands among the Ryukyu Islands (Mezaki 1980). During the Pleistocene, 1.5Ma - 0.01 Ma, the area of present Amami and Toku Islands formed the super-island (Kizaki & Oshiro 1980) and covered a large area where various genotypes could be maintained. Thus, many kinds of alleles of var. *australe* could have survived in the Amami and Toku Islands.

The mean value of A, P, He for each population of *Peucedanum japonicum* showed geographical cline of genetic diversities decreasing from the central Ryukyus northward (Fig. 4). The linear regression of A, P, He on latitude for the central Ryukyus and northward were significant (A, $R^2 = 0.37$, $P<0.05$; P, $R^2 = 0.46$, $P<0.05$; He, $R^2 = 0.28$, $P<0.05$). Allele frequencies for each population showed that northern populations, vars. *japonicum* and *latifolium* have the same allele at many polymorphic loci (Table 2). This shows that

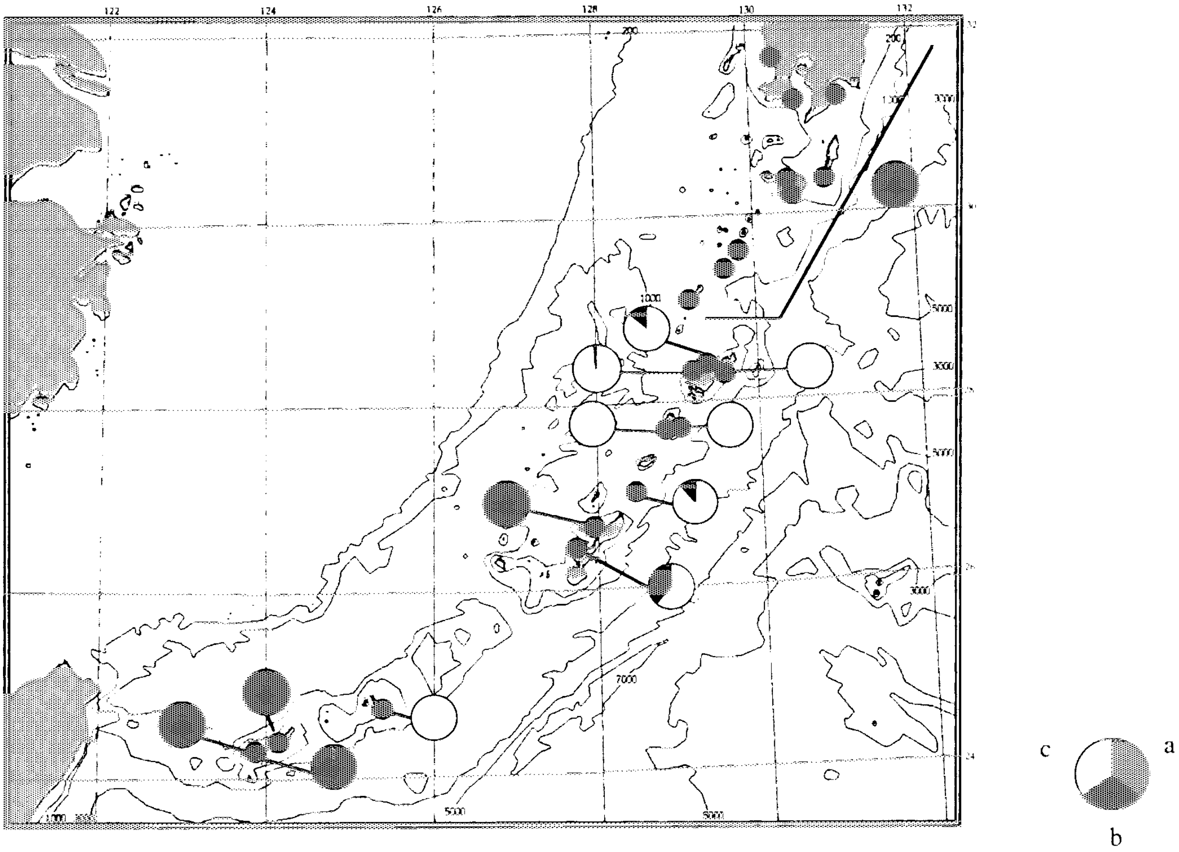

FIG. 3. Allele frequency of *Adh-2*.

the northern populations lost low frequency alleles by random genetic drift or migration from a population and recolonization of a small number of plants. It is not likely the same alleles at several loci were generated by mutation in several independent populations. Following, two scenarios are considered as an explanation for the geographical cline of genetic diversity for *P. japonicum*.

First, the northern populations of *Peucedanum japonicum* would lose alleles of low frequency by random genetic drift. Although var. *japonicum* population have different alleles at *Pgm-3*, the northern populations of *P. japonicum* have common dominant alleles (ex. *Adh-2b*, *Got-2c*, *Skd-b*) (Table 2). Therefore, low-frequency alleles were easily lost from a population by drift unless these alleles migrated from other populations. *Nm* estimated from *Fst* was as small as 0 (Table 5).

Second, through recolonization of a few populations, the northern populations of *Peucedanum japonicum* also have monomorphic alleles at different loci. During the Pleistocene Age, there were glacial advances and retreats in the Northern hemisphere (Nasu 1972). Palynological studies suggested that warm-temperate plants move southward during glacial ages (Kamei & Research group for the biogeography from Würm Glacial 1981, Tsukada 1984, Kuroda 1998). Present northern populations of *P. japonicum* would have shifted southward during a glacial period and recolonized after the glacial period. Further comparison of geographical patterns of genetic

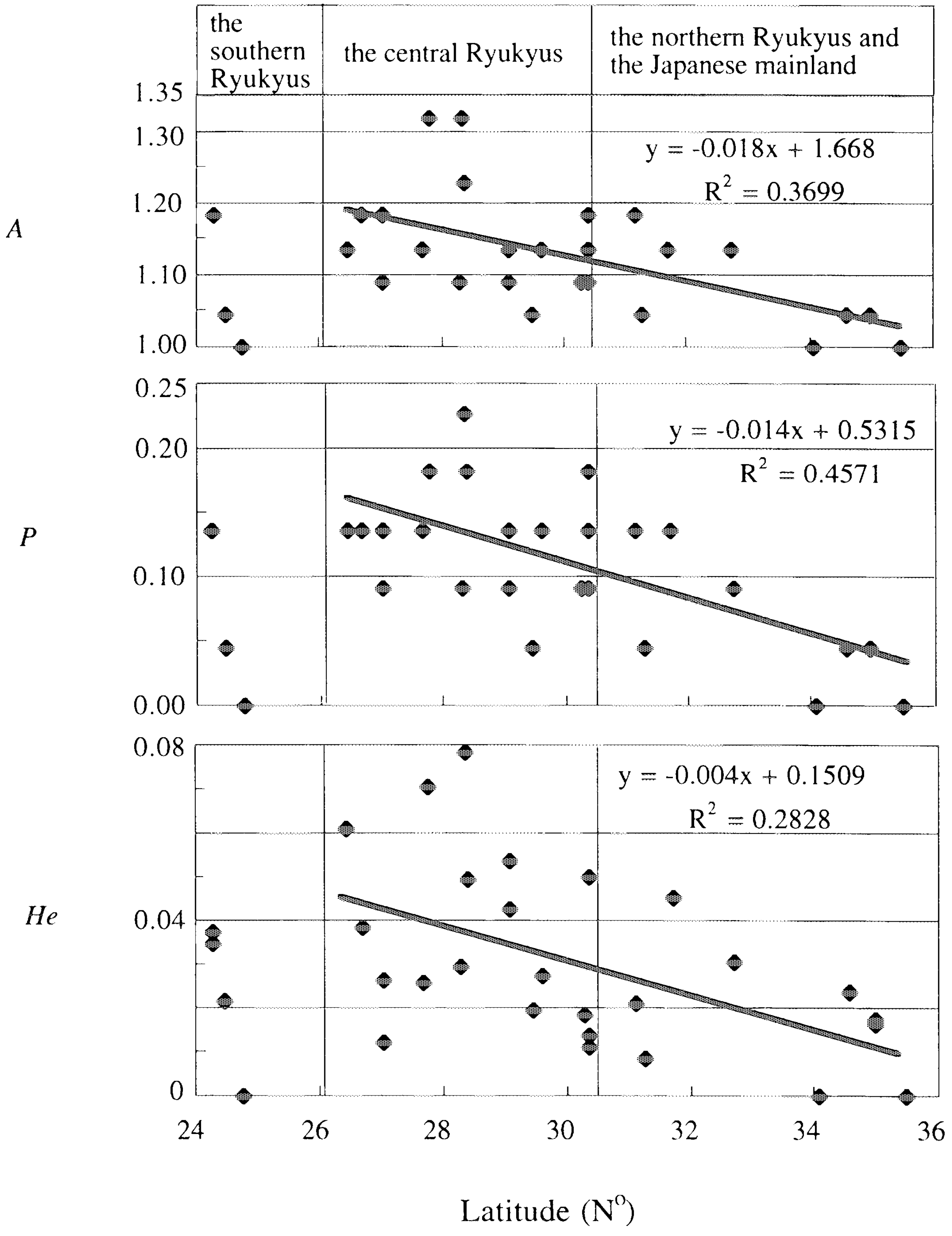

FIG. 4. Relationship between mean number of alleles per locus (*A*), the proportion of polymorphic loci (*P*), average expected heterozygosity (*He*) and latitude in 30 populations of *Peucedanum japonicum*.

differentiation between many independent plant taxa would provide more evidence to investigate these hypotheses.

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